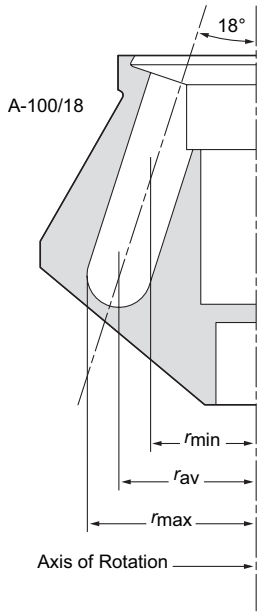
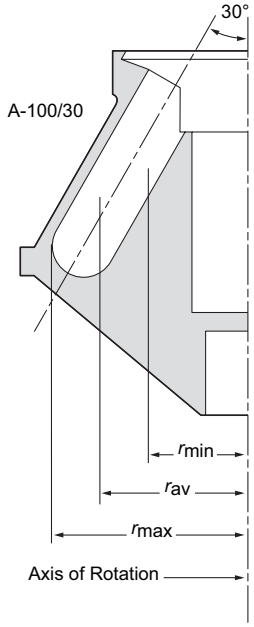
AF-TB-009AJ
February 2009

Instructions for Using the A-100/18 and A-100/30 Rotors

In the Beckman Coulter Airfuge® Ultracentrifuge



U.S. Pat. No. 3,997,104



SPECIFICATIONS

	A-100/18	A-100/30
Maximum speed (±5 000 rpm)	95 000 rpm	92 000 rpm
Air pressure at ultracentrifuge required for maximum speed	207 kPa (30 psig)	207 kPa (30 psig)
Density rating at maximum speed	1.2 g/mL	1.2 g/mL
Relative Centrifugal Field* at maximum speed		
At r_{max}	(14.7 mm) 149 000 × *g*	(17.6 mm) 167 000 × *g*
At r_{av}	(12.1 mm) 122 000 × *g*	(13.2 mm) 125 000 × *g*
At r_{min}	(9.5 mm) 96 000 × *g*	(8.8 mm) 83 400 × *g*
k factor at maximum speed	12	19
Number of tube cavities	6	6
Available tubes	see Table 1	see Table 1
Nominal tube dimensions	5 × 20 mm	5 × 20 mm
Nominal tube capacity	175 µL	240 µL
Nominal rotor capacity	1050 µL	1440 µL
Approximate acceleration time to maximum speed (rotor fully loaded)	1/2 to 1 min	1/2 to 1 min
Approximate deceleration time from maximum speed (rotor fully loaded)	3 1/2 min	3 1/2 min
Weight of fully loaded rotor	42 grams	44 grams
Rotor material	aluminum	aluminum

* Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed ($r\omega^2$) to the standard acceleration of gravity (g) according to the following formula:

$$\text{RCF} = \frac{r\omega^2}{g}$$

where r is the radius in millimeters, ω is the angular velocity in radians per second (2π RPM /60), and g is the standard acceleration of gravity (9807 mm/s^2). After substitution:

$$\text{RCF} = 1.12\, r \left(\frac{\text{RPM}}{1000}\right)^2$$

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DESCRIPTION

These Beckman Coulter rotors have been manufactured in a registered ISO 9001 or 13485 facility for use with the appropriately classified Beckman Coulter ultracentrifuge.

The A-100/18 and A-100/30 are fixed angle rotors. The A-100/18, rated for 95 000 rpm, has a tube angle of 18 degrees from the axis of rotation; the A-100/30 is rated for 92 000 rpm and has a tube angle of 30 degrees. These rotors, used with the Beckman Coulter Airfuge® Ultracentrifuge,[1] generate centrifugal forces for separating particles in a fraction of the time required by other ultracentrifuges. The high-efficiency A-100/18 is most useful for pelleting small particles, such as proteins and viruses, or for isolating microsomal fractions and plasma membranes. The A-100/30, with less reorientation of the contents, is more useful for sedimentation-equilibrium experiments for molecular weight determination. Both rotors can be used to clarify small volumes of lipemic serum by flotation of the chylomicrons. The rotors can each hold six tubes; up to 1050 µL of gradient and sample volume can be centrifuged per run in the A-100/18, and up to 1440 µL per run in the A-100/30.

The rotors are made of aluminum and are anodized for corrosion resistance—the A-100/18 is clear-anodized, and the A-100/30 is blue-anodized. Turbine flutes on each rotor allow it to be lifted and turned by jets of air. A white plastic bushing is fitted in the rotor bottom to aid rotor deceleration. Disposable rotor caps are made of polyethylene.

See the Warranty at the back of this manual for warranty information.

PREPARATION AND USE

Specific information about the A-100 rotors is contained here. Use the Airfuge Ultracentrifuge *instrument instruction manual (AF-IM) together with this manual for complete rotor and accessory operating instructions.*

WARNING

Normal operation may involve the use of solutions and test samples that are pathogenic, toxic, or radioactive. Operator error or tube failure may generate aerosols. Do not run toxic, pathogenic, or other hazardous materials in this rotor unless you take all appropriate safety precautions. Ask your laboratory safety officer to advise you about the level of containment required for your application and the proper decontamination or sterilization procedures to follow in the event that fluids escape from containers.

[1] U.S. Pat. Numbers 3,858,753 and 3,456,875

TUBES

Tubes that may be used in an A-100 rotor are listed in Table 1. Be sure to observe the maximum fill volumes shown, and use only those containers listed. Refer to *Chemical Resistances* (publication IN-175) for information on the chemical resistances of tube and accessory materials. Microliter-size tubes are generally not reusable.

Tubes listed in Table 1 have been centrifuge tested for use at temperatures between 2 and 25°C. Be sure that all tubes are thawed to at least 2°C prior to centrifugation. For centrifugation at other temperatures, tubes should be pretested under anticipated run conditions.

Containers may be filled less than or equal to the "Maximum Fill Volume" listed in the table. All tubes for a run must be filled to the same level. Fill tubes from the bottom to avoid air bubbles.

Table 1. Available Tubes for the A-100 Rotors.
Use only the items listed here and observe fill volumes.

Dimensions	Description	Part Number (pkg/100)	Maximum Fill Volume		Maximum Speed/RCF/k factor	
			A-100/18	A-100/30	A-100/18	A-100/30
5 × 20 mm	Ultra-Clear™	344718	175 µL	240 µL	95 000 rpm 149 000 × *g* 12	92 000 rpm 167 000 × *g* 19
5 × 20 mm	polyallomer	342630	175 µL	220 µL	95 000 rpm 149 000 × *g* 12	92 000 rpm 167 000 × *g* 19
5 × 20 mm	polyethylene	343622	100 µL	150 µL	95 000 rpm 149 000 × *g* 12	92 000 rpm 167 000 × *g* 19

ROTOR PREPARATION

NOTE

If solutes of low total concentration (≤1%) are pelleted, the sedimentation may be incomplete because the process is not entirely free of convection. To minimize convective disturbances, add sucrose to the solution, or perform the run in a cold room.

1. Before using the rotor, inspect the rotor bushing. Replace a worn bushing (refer to MAINTENANCE).

2. Use forceps to load the filled tubes into the rotor. If fewer than six tubes are being run, they must be arranged symmetrically in the rotor (see Figure 1). Opposing tubes must be filled to the same level with liquid of the same density.

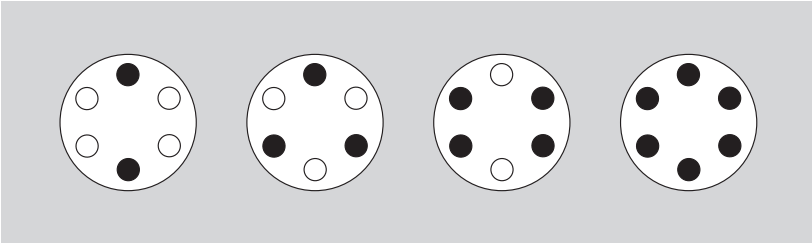

Figure 1. Arranging Tubes in the Rotor. Two, three, four, or six tubes can be centrifuged per run if they are arranged in the rotor as shown.

3. Install a rotor cap by rubbing it into place in the rotor groove. (See Figure 2.)

Figure 2. The Rotor Cap

CAUTION

Do not run the rotor without a cap. Use a new cap for each rotor use.

OPERATION

1. Keep a special stator pad for use with the 30° rotor only. Inspect it before *every* rotor use, and replace it if *any* shiny spots develop (refer to publication AF-IM). A worn pad is smooth and shiny on the upper half of the inside surface (Figure 3). A shiny pad has lost its cushioning effect and will eventually cause rotor failure.

2. Ensure that the rotor is clean and dry, then carefully place it in the ultracentrifuge.

3. Refer to the instrument instruction manual for ultracentrifuge operation.

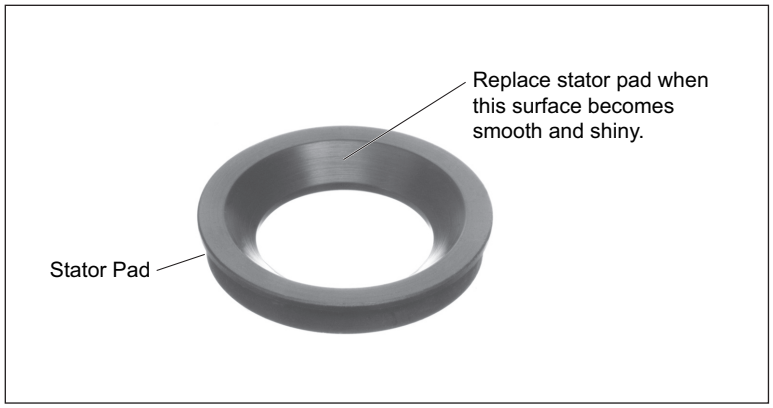

Figure 3. Ultracentrifuge Stator Pad

NOTE

Unusual noise during centrifugation is an indication of a missing rotor cap or load imbalance. Be sure a rotor cap is installed, and check the rotor for discrepancies in tube volumes or for asymmetrically placed tubes.

Rotor Wobble During Acceleration and Deceleration

Rotor wobble during acceleration can be corrected by *slowly* turning the pressure regulator knob to accelerate the rotor to the desired speed.

This light rotor decelerates quickly, and the normal 2-minute delay before the brake pin comes up to stabilize the rotor may be too long. If rotor wobble disturbs sample separation, reduce the delay period to about 1 minute 45 seconds (refer to the Airfuge® Ultracentrifuge Instruction Manual, AF-IM).

REMOVAL AND SAMPLE RECOVERY

CAUTION

If disassembly reveals evidence of leakage, you should assume that some fluid escaped the rotor. Apply appropriate decontamination procedures to the centrifuge and accessories.

1. Remove the rotor from the instrument.
2. Remove the rotor cap by pressing in the center of the cap to free an edge, then use forceps or your finger to grasp the cap. (See Figure 4.)

Figure 4. Removing the Rotor Cap. Press in the center of the cap to free an edge.

3. Remove the tubes, using forceps.
4. Recover the sample by one of the following methods:
   - Remove tube fractions by pipetting the supernatant liquid and then scraping out the pelleted material.
   - Separate liquid fractions by cutting the tubes with a razor blade—if handled carefully the fractions will be held in the tube parts by surface tension.

- Liquid fractions can be removed quickly and accurately using the Microtube Fractionator. With the Microtube Fractionator, fraction recovery can be repeated from tube to tube or sequentially in the same tube. Contact your Beckman Coulter representative for information about the Microtube Fractionator.

RUN TIMES

The *k* factor of the rotor is a measure of the rotor's pelleting efficiency. (Beckman Coulter has calculated the *k* factors for all of its preparative rotors at maximum rated speed and using full tubes.) The *k* factor is calculated from the formula:

$$k = \frac{\ln(r_{max} / r_{min})}{\omega^2} \times \frac{10^{13}}{3600} \quad (1)$$

where ω is the angular velocity of the rotor in radians per second ($\omega = 0.105 \times \text{rpm}$), r_{max} is the maximum radius, and r_{min} is the minimum radius.

After substitution:

$$k = \frac{(2.533 \times 10^{11}) \ln(r_{max} / r_{min})}{\text{rpm}^2} \quad (2)$$

Use the *k* factor in the following equation to estimate the run time *t* (in hours) required to pellet particles of known sedimentation coefficient *s* (in Svedberg units, *S*).

$$t = \frac{k}{s} \quad (3)$$

Run times can be estimated for centrifugation at less than maximum speed by adjusting the *k* factor as follows:

$$k_{adj} = k \left(\frac{\text{rated speed of rotor}}{\text{actual run speed}} \right)^2 \quad (4)$$

Run times can also be estimated from data established in prior experiments if the *k* factor of the previous rotor is known. For any two rotors, a and b:

$$\frac{t_a}{t_b} = \frac{k_a}{k_b} \quad (5)$$

For more information on *k* factors see *Use of* k *Factor for Estimating Run Times from Previously Established Run Conditions* (publication DS-719).

RUN SPEEDS

Run speed is a function of the air pressure applied to the ultracentrifuge (see Figure 5). Run speeds achieved at pressures between 138 and 207 kPa (20 and 30 psig) are recommended. Speeds achieved at pressures below 138 kPa (20 psig) may be satisfactory, but in some cases may result in rotor instability. If you experience difficulties in low-speed work, contact your Beckman Coulter representative.

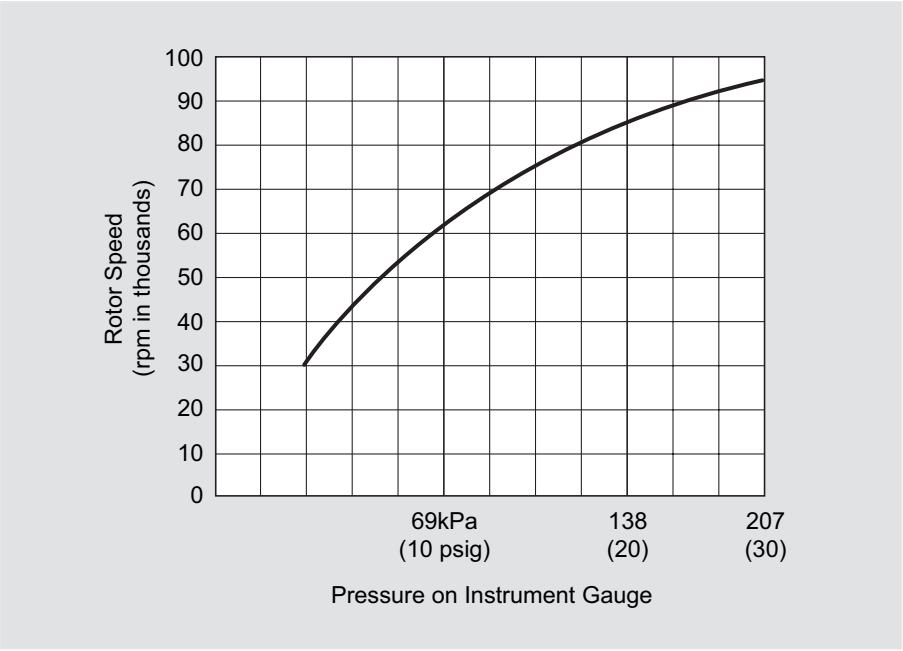

Figure 5. Rotor Speed vs. Air Pressure

The centrifugal force at a given radius in a rotor is a function of speed. Comparisons of forces between different rotors are made by comparing the rotors' relative centrifugal fields (RCF). When rotational speed is adjusted so that identical samples are subjected to the same RCF in two different rotors, the samples are subjected to the same force. The RCF at some rotor speeds is shown in Table 2.

Table 2. Relative Centrifugal Fields.
Entries in this table are calculated from the formula
$RCF = 1.12r (RPM/1000)^2$
and then rounded to three significant digits.

Air Pressure kPa (psig)	Rotor Speed (rpm)	Relative Centrifugal Field at r_{max} (× *g*)	
		A-100/18	A-100/30
207 (30)	95 000/92 000	149 000	167 000
186 (27)	90 000	133 000	155 000
152 (22)	85 000	119 000	142 000

CARE AND MAINTENANCE

MAINTENANCE

NOTE

Do not use sharp tools on the rotor. Scratches in the anodized surface could lead to corrosion.

Regularly inspect the rotor bushing. Replace worn or damaged bushings as follows:

1. Remove the old bushing, using one tip of a forceps. Be careful not to scratch the rotor.
2. Place a new bushing (339639) into the rotor, beveled edge first.
3. Push the bushing in until it is flush with the rotor bottom. Lightly press the rotor against a flat surface to be sure the bushing is properly installed.
4. Perform a trial run with the fully assembled rotor.

Store the rotor in a dry environment (not in the instrument) with the lid removed. Refer to *Chemical Resistances* for the chemical compatabilities of rotor and tube materials. Your Beckman Coulter representative provides contact with the Field Rotor Inspection Program and the rotor repair center.

CLEANING

Wash the rotor and rotor components immediately if salts or other corrosive materials are used or if spillage has occurred. Do not allow corrosive materials to dry on the rotor.

Under normal use conditions, wash the rotor at least weekly to prevent buildup of residues.

NOTE

Do not wash rotor components in a dishwasher. Do not soak in detergent solution for long periods, such as overnight.

1. Wash the rotor using a mild detergent such as Beckman Solution 555™ (339555), that won't damage the rotor. Dilute the detergent 10 to 1 with water. Clean the rotor groove and the tube cavities with a cotton-tipped swab.
2. Rinse thoroughly with distilled water.
3. Air-dry the rotor upside down. *Do not use acetone to dry the rotor.*

DECONTAMINATION

If the rotor (and/or accessories) becomes contaminated with radioactive material, decontaminate it using a solution which will not damage the anodized surfaces. Beckman Coulter has tested a number of solutions and found two that do not harm anodized aluminum: RadCon Surface Spray or IsoClean Solution (for soaking),[2] and Radiacwash.[3]

While Beckman Coulter has tested these methods and found that they do not damage components, no guarantee of sterility or disinfection is expressed or implied. Consult your laboratory safety officer regarding the proper decontamination methods to use.

If the rotor or other components are contaminated with toxic or pathogenic materials, follow appropriate decontamination procedures as outlined by your laboratory safety officer.

[2] In U.S.A., contact Nuclear Associates (New York); in Eastern Europe and Commonwealth States, contact Victoreen GmbH (Munich); in South Pacific, contact Gammasonics Pty. Ltd. (Australia); in Japan, contact Toyo Medic Co. Ltd. (Tokyo).

[3] In U.S.A., contact Biodex Medical Systems (Shirley, NY); internationally, contact the U.S. office to find the dealer nearest you.

STERILIZATION AND DISINFECTION

- The rotor can be autoclaved at 121°C for about 30 minutes. Place the rotor in the autoclave upside down, without a cap.
- Ethanol (70%)[4] may be used on all rotor components.
- The microliter-size tubes and rotor caps are disposable.

While Beckman Coulter has tested these methods and found that they do not damage the rotor or components, no guarantee of sterility or disinfection is expressed or implied. When sterilization or disinfection is a concern, consult your laboratory safety officer regarding proper methods to use.

[4] Flammability hazard. Do not use in or near an operating ultracentrifuge.

RETURNING A ROTOR

Before returning a rotor or accessory for any reason, prior permission (a Returned Goods Authorization form) must be obtained from Beckman Coulter, Inc. This RGA form may be obtained from your local Beckman Coulter sales office. It should contain the following information:

- serial number,
- history of use (approximate frequency of use),
- reason for the return,
- original purchase order number, billing number, and shipping number, if possible,
- name and phone number of the person to be notified upon receipt of the rotor or accessory at the factory, and
- name and phone number of the person to be notified about repair costs, etc.

To protect our personnel, it is the customer's responsibility to ensure that the parts are free from pathogens and/or radioactivity. Sterilization and decontamination must be done before returning the parts. Smaller items (such as tubes, bottles, etc.) should be enclosed in a sealed plastic bag.

All parts must be accompanied by a note, plainly visible on the outside of the box or bag, stating that they are safe to handle and that they are not contaminated with pathogens or radioactivity. ***Failure to attach this notification will result in return or disposal of the items without review of the reported problem***.

Use the address label printed on the RGA form when mailing the rotor and/or accessories.

Customers located outside the United States should contact their local Beckman Coulter office.

SUPPLY LIST

NOTE

Publications referenced in this manual can be obtained by calling Beckman Coulter at 1-800-742-2345 in the United States, or by contacting your local Beckman Coulter office.

Contact Beckman Coulter Sales (1-800-742-2345 in the United States; worldwide offices are listed on the back cover of this manual) or see the *Ultracentrifuge Rotors, Tubes, & Accessories* catalog (BR-8101) for detailed information on ordering parts and supplies. For your convenience, a partial list is given below.

REPLACEMENT ROTOR PARTS

A-100/18 rotor assembly	347593
A-100/30 rotor assembly	347594
Rotor base bushing	339639
Rotor and tube rack	342707
Rotor stand/vise	341252
Stator pad	339610

OTHER

Tubes	see Table 1
Tube rack for 5-mm diameter tubes	348301
Tube cap strip, 6-place (pkg/50)	343773
Rotor caps (pkg/20)	339643
Beckman Solution 555 (1 qt)	339555
Rotor cleaning brush	339379

ULTRACENTRIFUGE ROTOR WARRANTY

All Beckman Coulter ultracentrifuge Fixed Angle, Vertical Tube, Near Vertical Tube, Swinging Bucket, and Airfuge rotors are warranted against defects in materials or workmanship for the time periods indicated below, subject to the Warranty Conditions stated below.

Preparative Ultracentrifuge Rotors . . . . . 5 years — No Proration

Analytical Ultracentrifuge Rotors. . . . . 5 years — No Proration

ML and TL Series Ultracentrifuge Rotors . . . . . 5 years — No Proration

Airfuge Ultracentrifuge Rotors . . . . . 1 year — No Proration

For Zonal, Continuous Flow, Component Test, and Rock Core ultracentrifuge rotors, see separate warranty.

Warranty Conditions (as applicable)

1) This warranty is valid for the time periods indicated above from the date of shipment to the original Buyer by Beckman Coulter or an authorized Beckman Coulter representative.

2) This warranty extends only to the original Buyer and may not be assigned or extended to a third person without written consent of Beckman Coulter.

3) This warranty covers the Beckman Coulter Centrifuge Systems only (including but not limited to the centrifuge, rotor, and accessories) and Beckman Coulter shall not be liable for damage to or loss of the user's sample, non-Beckman Coulter tubes, adapters, or other rotor contents.

4) This warranty is void if the Beckman Coulter Centrifuge System is determined by Beckman Coulter to have been operated or maintained in a manner contrary to the instructions in the operator's manual(s) for the Beckman Coulter Centrifuge System components in use. This includes but is not limited to operator misuse, abuse, or negligence regarding indicated maintenance procedures, centrifuge and rotor classification requirements, proper speed reduction for the high density of certain fluids, tubes, and tube caps, speed reduction for precipitating gradient materials, and speed reduction for high-temperature operation.

5) Rotor bucket sets purchased concurrently with or subsequent to the purchase of a Swinging Bucket Rotor are warranted only for a term co-extensive with that of the rotor for which the bucket sets are purchased.

6) This warranty does not cover the failure of a Beckman Coulter rotor in a centrifuge not of Beckman Coulter manufacture, or if the rotor is used in a Beckman Coulter centrifuge that has been modified without the written permission of Beckman Coulter, or is used with carriers, buckets, belts, or other devices not of Beckman Coulter manufacture.

7) Rotor parts subject to wear, including but not limited to rotor O-rings, VTi, NVT™, TLV, MLN, and TLN rotor tube cavity plugs and gaskets, tubing, tools, optical overspeed disks, bearings, seals, and lubrication are excluded from this warranty and should be frequently inspected and replaced if they become worn or damaged.

8) Keeping a rotor log is not mandatory, but may be desirable for maintenance of good laboratory practices.

Repair and Replacement Policies

1) If a Beckman Coulter rotor is determined by Beckman Coulter to be defective, Beckman Coulter will repair or replace it, subject to the Warranty Conditions. A replacement rotor will be warranted for the time remaining on the original rotor's warranty.

2) If a Beckman Coulter centrifuge is damaged due to a failure of a rotor covered by this warranty, Beckman Coulter will supply free of charge (i) all centrifuge parts required for repair (except the drive unit, which will be replaced at the then current price less a credit determined by the total number of revolutions or years completed, provided that such a unit was manufactured or rebuilt by Beckman Coulter), and (ii) if the centrifuge is currently covered by a Beckman Coulter warranty or Full Service Agreement, all labor necessary for repair of the centrifuge.

3) If a Beckman Coulter rotor covered by this warranty is damaged due to a malfunction of a Beckman Coulter ultracentrifuge covered by an Ultracentrifuge System Service Agreement, Beckman Coulter will repair or replace the rotor free of charge.

4) If a Beckman Coulter rotor covered by this warranty is damaged due to a failure of a Beckman Coulter tube, bottle, tube cap, spacer, or adapter, covered under the Conditions of this Warranty, Beckman Coulter will repair or replace the rotor and repair the instrument as per the conditions in policy point (2) above, and the replacement policy.

5) Damage to a Beckman Coulter rotor or instrument due to the failure or malfunction of a non-Beckman Coulter tube, bottle, tube cap, spacer, or adapter is not covered under this warranty, although Beckman Coulter will assist in seeking compensation under the manufacturer's warranty.

Disclaimer

IT IS EXPRESSLY AGREED THAT THE ABOVE WARRANTY SHALL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND OF THE WARRANTY OF MERCHANTABILITY AND BECKMAN COULTER, INC. SHALL HAVE NO LIABILITY FOR SPECIAL OR CONSEQUENTIAL DAMAGES OF ANY KIND WHATSOEVER ARISING OUT OF THE MANUFACTURE, USE, SALE, HANDLING, REPAIR, MAINTENANCE, OR REPLACEMENT OF THE PRODUCT.

Factory Rotor Inspection Service

Beckman Coulter, Inc., will provide free mechanical and metallurgical inspection in Indianapolis, Indiana, USA, of any Beckman Coulter rotor at the request of the user. (Shipping charges to Beckman Coulter are the responsibility of the user.) Rotors will be inspected in the user's laboratory if the centrifuge in which they are used is covered by an appropriate Beckman Coulter Service Agreement. Contact your local Beckman Coulter office for details of service coverage or cost.

Before shipping, contact the nearest Beckman Coulter Sales and Service office and request a Returned Goods Authorization (RGA) form and packaging instructions. Please include the complete rotor assembly, with buckets, lid, handle, tube cavity caps, etc. A SIGNED STATEMENT THAT THE ROTOR AND ACCESSORIES ARE NON-RADIOACTIVE, NON-PATHOGENIC, NON-TOXIC, AND OTHERWISE SAFE TO SHIP AND HANDLE IS REQUIRED.

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